STRONG Insights

How Zebrafish Are Shaping the Future of Bone and Muscle Research

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Used in scientific research since the 1980s, zebrafish (Danio rerio) have evolved into a strong contender in translational studies. At the University of Malta, Prof. Melissa M. Formosa and her team are utilising this model for Project STRONG, a research initiative centred on osteosarcopenia – the degenerative concurrent loss of bone and muscle.

The origins of Project STRONG are rooted in Project ZeEBRA (R&I-2019-018T), a three-year initiative that laid the groundwork for establishing zebrafish as a viable biomedical model locally, as well as a housing facility for them. Initially focused on osteoporosis, the ZeEBRA team developed and optimised drug toxicity testing protocols. Research Support Officer Margherita Muscat explains that these early years were challenging because the team was developing approaches without well-established protocols. Standardised zebrafish embryo protocols typically only extend to four days, before significant bone mineralisation occurs. To study skeletal outcomes, the team had to extend the experimental window to seven days, optimising variables such as multiwell size, fish density, and media volume to achieve reliable, reproducible results. Prof. Melissa M. Formosa notes that this validated methodology became the 'backbone' of STRONG, allowing the team to move directly into compound testing with a robust experimental framework.

WHY ZEBRAFISH?

Setting up a new model organism is a rather demanding task. Formosa gained training on zebrafish in the Netherlands, but replicating those systems in Malta required significant adaptation. Research Support Officer Dr Sherif S. Suleiman notes that variables such as water conditions and temperature play a critical role. Ultimately, keeping the fish 'happy' through optimal environmental conditions is the foundation for reliable results. Once established, the advantages of zebrafish are immediate. Their high fecundity allows for large numbers of embryos to be generated, enabling statistically powerful experiments within a short timeframe.

Zebrafish develop rapidly, and by five days post fertilisation, most organ systems are formed. This short developmental timeline allows many experiments to generate results within one week, substantially reducing experimental time compared with many mammalian models. During early development, the embryos are optically transparent, which enables direct observation of internal biological processes *in vivo* without the need for invasive procedures. Zebrafish also share considerable genetic similarity with humans, with around 70% of human genes having at least one zebrafish orthologue. Although they do not possess limbs, zebrafish are widely used to investigate skeletal biology, including fracture repair and regeneration, in part because of their capacity to regenerate fins and scales.

OSTEOSARCOPENIA: THE DUAL THREAT

Osteosarcopenia represents two age-related conditions: osteoporosis (loss of bone mass with increased susceptibility to bone fractures) and sarcopenia (reduced skeletal muscle mass and strength). Formosa explains that while osteoporosis is widely studied, there is a lack of osteoanabolic therapies that actively build new bone, as most current treatments are antiresorptives that merely slow bone loss. Sarcopenia, meanwhile, lacks any approved pharmacological treatment.

Emerging research shows that bone and muscle are biologically interconnected, sharing molecular

From left to right: Dr Sherif S. Suleiman, Margherita Muscat, Prof. Melissa M. Formosa, Amy Marie Vella, Matthew Camilleri
Photo by Kristov Scicluna

signalling pathways. As populations age, the simultaneous decline of both tissues increases the risk of frailty and fractures. Identifying therapeutic targets that strengthen both tissues at once is now a global priority.

THE CHEMICAL LIBRARY: NATURAL VS SYNTHETIC

A central pillar of Project STRONG is the diversity of compounds being screened, including natural, semi-synthetic, and fully synthetic products. Muscat notes that to date, approximately a dozen candidates have been screened, with several showing promise in enhancing bone mineralisation. Each category presents its own unique advantages and disadvantages. Natural products, for example, often found in existing supplements, offer a potentially faster route to market, though they present challenges regarding solubility and stability in an aquatic environment, Suleiman notes. Synthetic libraries are readily available and stable, but carry a higher risk of toxicity. Formosa describes the process as a 'learning curve' in which the safety profile and outcome shift depending on the individual product's chemical properties.

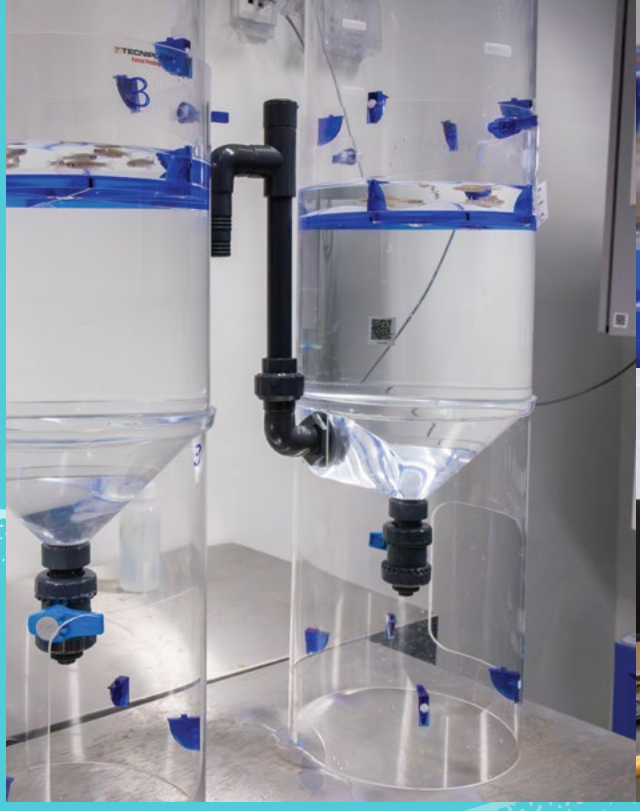
VISUALISING THE DATA: BONE AND MUSCLE

Muscat explains that for bone studies, zebrafish larvae are exposed to compounds from three to seven days post fertilisation. To maintain stable dosing and prevent toxicity, half the medium is refreshed daily. On Day 7, larvae are stained to visualise the operculum, which is the bony plate proximal to the gills that serves as a reliable readout of skeletal development. Several treated larvae have shown increased mineralisation compared to controls, suggesting true osteoanabolic potential.

The focus is now expanding to the muscles. Using polarised light microscopy, researchers can visualise muscle fibre integrity in live, sedated larvae. Since the larvae are transparent, differences in muscle structure can be assessed non-invasively. Functional analysis adds another layer of insight. Using visual tracking technology (known as the DanioVision), individual larvae are placed in multiwell plates to record velocity, total distance travelled, and movement patterns. Light- and sound-based stimuli can also be introduced to trigger startle responses, providing data on neuromuscular integrity and neurotoxicity. Together, these parameters generate a detailed picture of muscle performance and coordination.

BRIDGING THE GAP TO HUMAN HEALTH

Formosa explains that while direct extrapolation from fish to humans is complex, the team validates their findings using positive controls. They frequently use bisphosphonates – a class of prescription medications used for treating osteoporosis. When these existing drugs produce the expected effect in zebrafish, it validates the model's relevance to human physiology. The sheer scale of



Zebrafish spawning systems designed to effectively collect large numbers of embryos (left)
Wild-type (AB) zebrafish (top right)
The DanioVision is designed to track the activity and movement patterns of zebrafish (bottom right)
Photos by Kristov Scicluna

zebrafish research also adds weight to the findings. The ability to test compounds across large sample sizes provides a level of statistical power that is often cost-prohibitive with other traditional models. As Suleiman notes, this makes the results far more robust for future translational steps.

BEYOND STRONG: INTERNATIONAL IMPACT, DIABETES AND OSTEOARTHRITIS

Project STRONG's influence has reached beyond the Maltese Islands. The natural compounds under investigation are supplied by Prof. Attila Hunyadi, from the University of Szeged in Hungary, who is interested in their effects on muscle, where pharmacological options remain limited. The strength of these findings has already sparked discussions regarding intellectual property and potential patenting.

Furthermore, the team's success has led to the launch of Project ZEDCOM (Zebrafish Evaluation of Diabetes COMorbidities and drug targets), which is financed by the Research Excellence Fund of the University of Malta. Muscat explains that the progression into diabetes research was a natural one, driven by the high rates of diabetes in Malta and the shared signalling pathways involved in osteoporosis, sarcopenia, and diabetes. Additionally, the zebrafish's incredible regenerative properties make it an excellent model for studying wound healing – a critical component of diabetes care.

Building on the foundations of Project STRONG and the earlier ZeEBRA work, the team is now moving into an exciting new collaboration through a TÜBITAK grant, known as OSTEOBOOST. This initiative continues the group's established expertise in zebrafish-based drug testing, but with an expanded focus beyond bone alone. OSTEOBOOST will explore compounds targeting both bone and cartilage, addressing the overlapping burden of osteoporosis and osteoarthritis, which are two of the most common musculoskeletal conditions affecting older adults.

Therefore, through these integrated projects, the Project STRONG research team is not simply studying individual diseases, but the complex, interconnected nature of human ageing and metabolic health.

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